Influence of single nucleotide polymorphisms in factor VIII and von Willebrand factor genes on plasma factor VIII activity: the ARIC Study

Marco Campos

Ashley L. Buchanan
University of Rhode Island, buchanan@uri.edu

See next page for additional authors

Follow this and additional works at: https://digitalcommons.uri.edu/php_facpubs

Terms of Use
All rights reserved under copyright.

Citation/Publisher Attribution
Available at: http://dx.doi.org/10.1182/blood-2011-10-383661
Influence of single nucleotide polymorphisms in factor VIII and von Willebrand factor genes on plasma factor VIII activity: the ARIC Study

Marco Campos, Ashley Buchanan, Fulu Yu, Maja Barbatic, Yang Xiao, Lloyd E. Chambless, Kenneth K. Wu, Aaron R. Folsom, Eric Boerwinkle, and Jing-fei Dong

Cardiovascular Sciences Section, Department of Medicine, Baylor College of Medicine, Houston, TX; Department of Biostatistics, University of North Carolina, Chapel Hill, NC; Human Genome Sequencing Center, Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, TX; Human Genetic Center, The University of Texas School of Public Health, Houston, TX; Puget Sound Blood Center, Seattle, WA; National Health Research Institutes, Taipei, Taiwan; and Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN

Factor VIII (FVIII) functions as a cofactor for factor IXa in the contact coagulation pathway and circulates in a protective complex with von Willebrand factor (VWF). Plasma FVIII activity is strongly influenced by environmental and genetic factors through VWF-dependent and -independent mechanisms. Single nucleotide polymorphisms (SNPs) of the coding and promoter sequence in the FVIII gene have been extensively studied for effects on FVIII synthesis, secretion, and activity, but impacts of non-disease-causing intrinsic SNPs remain largely unknown. We analyzed FVIII SNPs and FVIII activity in 10,434 healthy Americans of European (EA) or African (AA) descent in the Atherosclerosis Risk in Communities (ARIC) study. Among covariates, age, race, diabetes, and ABO contributed 2.2%, 3.5%, 4%, and 10.7% to FVIII intersubject variation, respectively. Four intrinsic FVIII SNPs associated with FVIII activity and 8 with FVIII-VWF ratio in a sex- and race-dependent manner. The FVIII haplotypes AT and GCTTTT also associated with FVIII activity. Seven VWF SNPs were associated with FVIII activity in EA subjects, but no FVIII SNPs were associated with VWF Ag. These data demonstrate that intrinsic SNPs could directly or indirectly influence intersubject variation of FVIII activity. Further investigation may reveal novel mechanisms of regulating FVIII expression and activity. (Blood. 2012;119(8):1929-1934)

Introduction

Factor VIII (FVIII) functions as a cofactor for factor IXa in the contact coagulation pathway and is synthesized as a single-chain precursor with a domain structure of n-A1-A2-B-A3-C1-C2-c.1,2 It is secreted as a noncovalent heterodimer produced from intracellular cleavage at 2 peptide bonds in the B domain: Arg1313-Ala1314 and Arg1648-Glu1649, generating a heavy (A1, A2, and a part of B domains) and a light chain (A3, C1, and C2 domains).2,3 FVIII is synthesized in multiple cell types, but hepatic sinusoid endothelial cells appear to be a main source of circulating FVIII.4,5 FVIII is highly sensitive to proteolysis and is protected by forming a high-affinity complex (Kd = 0.2-0.5nM) with VWF in the circulation.6,7 The half-life of VWF-bound FVIII is significantly longer than plasma FVIII from patients lacking VWF (type III VWD).8,9 This FVIII-VWF complex is formed primarily through an interaction between the light chain of FVIII10 and the D’ and D3 domains of VWF.11,13 FVIII is activated to FVIIIa when it is cleaved by thrombin at A1-A2 and A2-B junctions in the heavy chain and in the N-terminal region of the light chain,3 whereas cleavage in the light chain releases FVIIa from VWF.14

The molar ratio of circulating FVIII and VWF is ~1:50 of FVIII to VWF monomer.6 However, the 2 molecules can form a complex in a 1:1 stoichiometry in vitro, suggesting that all FVIII-binding sites on a VWF multimer are available and the FVIII-VWF ratio may primarily depend on the amount of circulating FVIII.6,7 The baseline level of FVIII varies considerably among individuals and increase further in conditions such as inflammation.15 This variation is caused by multiple factors, but genetic influence is significant. One major genetic factor is ABO blood types,16,17 which affects FVIII activity primarily because of ABO impact on VWF.18 However, a small, but significant, VWF-independent ABO effect on FVIII is also reported in healthy subjects.19

The FVIII gene spans 186 kb on chromosome X (q28) and contains 26 exons that range from 69 bp to 3.1 kb in size.20,21 FVIII introns vary from 0.2 kb for intron 17 to 32 kb for intron 22. Within intron 22, there are 2 distinct non-FVIII gene elements controlled by a bidirectional promoter.22,23 The FVIII mRNA is ~9 kb that encodes a mature glycoprotein of 2332 aa without known alternatively spliced forms.2 The FVIII locus is polymorphic with a majority of single nucleotide polymorphisms (SNPs) located in introns,24-25 but impacts of these intrinsic and non-disease-causing SNPs on plasma FVIII activity remain largely unknown. In this study, we correlated 19 FVIII SNPs with FVIII activity measured in plasma samples from 10,434 healthy American subjects of European (EA, 8056) or African (AA, 2378) descent in the Atherosclerosis Risk in Communities (ARIC) Study. In addition, 75 VWF SNPs that we have previously studied for their impact on VWF Ag26 were also analyzed for their effect on FVIII activity.

Methods

Study population

ARIC is a prospective cohort study designed to assess atherosclerosis, clinical atherosclerotic diseases, and cardiovascular risk factors. Baseline


M.C., A.B., and F.Y. contributed equally.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 USC section 1734.

© 2012 by The American Society of Hematology
samples and demographic data were collected from 15 792 subjects of 45 to 64 years of age using probability sampling from Forsyth County, NC; Jackson, MS; the northwestern suburbs of Minneapolis, MN; and Washington County, MD from 1987 to 1989 (http://www.cscc.unc.edu/aric/). This study included Americans of EA and AA descent. Subjects who did not consent to share genetic information were excluded from the study (N = 45). The use of these data were approved by the institutional review boards of all participating institutions for the ARIC study.

Baseline measurements
Blood was drawn from an antecubital vein from subjects who were asked to arrive at clinic after fasting at least 12 hours. FVIII activity was measured by the ability of a testing sample to correct clotting time of human FVIII-deficient plasma and reported as a percentage (George King Biomedical Inc). VWF Ag was determined by a commercial ELISA kit (American Bioproducts) and reported as a percentage of the Universal Coagulation Reference Plasma (Thromboscreen, Pacific Hemostasis; Curtin Matheson Scientific Inc). The reliability coefficient (1 – intraindividual variance/total variance) obtained from repeat testing of individuals over several weeks was 0.68 for VWF and 0.86 for FVIII. Age, race, sex, body mass index (BMI), hypertension, diabetes, ever smoking status, and ABO genotype were adjusted for their known impacts on VWF and FVIII.27,28

SNP genotyping and haplotype construction
SNPs genotyped on an Affymetrix 6.0 platform in the region encompassing the FVIII gene (153 717 264 to 153 904 192 on X chromosome) and VWF gene (5 928 997-6 098 180 on chromosome 12) were included in the data analyses. A total of 19 FVIII and 75 VWF SNPs were analyzed for the study (supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article), where letters A and B indicate major and minor alleles. We also used the fastPHASE 1.2 program to resolve haplotypes from the unphased SNP genotype data and Haploview to determine regions in strong linkage disequilibrium (LD), their cosegregation ratios, and underlying haplotypes in each region.26,29

Because of known associations between O blood type and plasma VWF levels and FVIII activity, blood type O was genotyped based on a single base deletion (G) at nt 261 (RS8176719) that shifts the reading frame to generate a premature termination codon. The truncated enzyme is unable to transfer a sugar moiety to the H Ag. This SNP was therefore used to distinguish between type O (homozygous for nt261) and type non-O subjects in the analysis (nt261 was on one allele or absent).30

Data analysis
All SNPs were evaluated for Hardy-Weinberg Equilibrium (HWE) by \( \chi^2 \) test statistics or exact tests, as appropriate. HWE was tested for each race-by-sex level separately. The majority of FVIII SNPs were in HWE for EA women except RS5987068 and RS5987061, which did not have enough variation to test HWEs, and RS12392769, which was not in HWE for AA women. Because the FVIII gene is on the X chromosome, tests of HWE were irrelevant for men. Statistical significance was determined at the level of \( P < .0005 \) using a Bonferroni adjustment for multiple comparisons, which was calculated based on a formula of 0.05 divided by a total of 94 SNPs included in analysis.

Linear models were used to evaluate the association of FVIII activity with each FVIII SNP. The analysis was stratified on race and sex. The models were adjusted for age, hypertension, diabetes, BMI, ever smoking, and ABO. For haplotype analyses at each block of interest, a numerical variable was assigned for each common haplotype by counting the frequency (0, 1, or 2) of the haplotype for women, but there are only 2 possible levels of the haplotype variable (0 or 1) for men because the FVIII gene is on the X chromosome. Linear models were also used to assess the association between FVIII or VWF plasma levels and haplotype frequencies.

Results
Among the 15 792 ARIC participants of EA and AA descent, 10 434 were included in the analyses after exclusion of subjects for lacking data on (1) FVIII activity, VWF Ag, or both (n = 280); (2) SNPs on both genes (n = 3032); or (3) ABO genotypes (n = 1898). Demographics for the 10 434 subjects are displayed in supplemental Table 2. There were significant differences in age, BMI, and smoking status between the 4 race-by-sex groups. EA subjects were older and have a lower BMI, compared with AA subjects, for both sexes. More men were in ever-smoking status than women were for both races. AA subjects had higher prevalences of diabetes and hypertension compared with EA subjects.

FVIII activity and association with VWF Ag levels
The mean plasma FVIII activity and VWF Ag levels were 127.5% (SD = 37.3 and median at 123%) and 113.5% (SD = 45.2 and median at 106%), respectively. Both mean values were > 100% for reasons to be further determined. One possibility is the inclusion of a large number of AA subjects who are known to have higher FVIII and VWF. Both measurements varied considerably among the cohort subjects (20%-540% for FVIII and 22%-412% for VWF, Figure 1). The FVIII-VWF ratio ranged from 0.18 to 3.73 with a mean ratio of 1.20. The correlation coefficient between FVIII activity and VWF Ag were 0.70 (women) and 0.69 (men) for EA subjects and 0.69 (women) and 0.74 (men) for AA subjects, respectively. FVIII activity and VWF Ag were significantly different among the 4 race-by-sex groups. FVIII activity was higher for women, compared with men (for both race), and for AA subjects, compared with EA subjects. AA subjects also had a higher VWF Ag, compared with EA subjects. The FVIII/VWF ratio was higher in women, compared with men, but was comparable between the race groups (Table 1). When both measurements were grouped in

---

Figure 1. Distributions of FVIII activity, VWF Ag, and FVIII-VWF ratio in 10 434 subjects included in the study.
quantiles, FVIII activity was found in parallel with VWF Ag in 39% to 66% of subjects, whereas up to 27% mismatched. Among these mismatched, 2% of subjects had a significant split where a very high VWF Ag (fourth quartile) was paired with a very low FVIII activity (first quartile) or vice versa (Table 2). This split between the 2 measurements varied from 1% to 3% in the 4 race-by-sex groups (supplemental Table 3).

There were 5005 subjects (48%) who are the O blood type and 5429 subjects (52%) who are non-O blood type. ABO genotype accounted for 10.7% of FVIII variation when it was analyzed together with nongenetic covariates in a linear model (supplemental Table 4). Among the nongenetic covariates used for adjustment, race, diabetes, and age accounted for 4.0%, 3.5%, and 2.2% of the variance, respectively.

### SNP and haplotype correlation with FVIII activity

There were 19 FVIII SNPs available in the ARIC genome-wide association study (GWAS) database. Eighteen of them were intronic (94.7%) and 1 was nonsynonymous from exon 26 (RS1800297) that changes a methionine residue at the position of 2257 to a valine residue (M2257V). Seventy-five VWF SNPs were also analyzed for their effect on FVIII activity. Among them, 70 were intronic (93.3%) and 5 exonic (6.7%), but all coding SNPs were synonymous. Among the 19 FVIII SNPs, RS6643714 was significantly associated with FVIII activity after adjustment for age, sex, smoking status, diabetes, and hypertension. A further adjustment with ABO yielded 3 additional SNPs that were significantly associated with FVIII activity (Table 3). The association of these 4 SNPs with FVIII activity was found in EA men, but not in EA women and AA subjects of either sex. All 4 SNPs were also associated with the FVIII-VWF ratio, but again in EA men only (Table 4). In addition, 4 new SNPs were associated with the FVIII-VWF ratio in EA subjects of either sex, but not in AA subjects.

The large ARIC database allowed us to construct FVIII haplotypes that included rare alleles to identify specific SNPs that are transmitted together and to determine whether their effects are additive. Using the fastPHASE 1.2 program, we identified 2 major haplotype blocks that are in linkage disequilibrium in the FVIII gene and accounted for 82.4%, 73.5%, 83.6%, and 74.3% of all haplotypes for EA women, AA women, EA men, and AA men, respectively (Figure 2). SNPs in each LD haplotype were identical for male and female subjects, but varied significantly between AA and EA subjects (Table 5), indicating a highly diverse coaggregation of SNPs between EA and AA subjects. Among the haplotypes, AT in block 1 and GCTTTT in block 2 were associated with FVIII activity in EA men after adjustment for age, BMI, ever smoking, diabetes, hypertension, and ABO (Table 6).

### Cross-genetic influence between FVIII and VWF SNPs

Finally, we examined cross-influence between the 2 measurements and found no FVIII SNPs associated with VWF Ag levels before or after adjustment for ABO and other covariates. In contrast, 5 and 2 VWF SNPs were associated with FVIII activity for EA women and EA men, respectively (Table 7), but not for AA subjects.
Models are adjusted for age, BMI, ever smoking, diabetes, and hypertension (1 subject with outlier was not included from these models).

SNP indicates single nucleotide polymorphism; and EA, Americans of European descent.

*There were 37 men were missing information on at least 1 of the predictors and were excluded from these models.
†There were 27 women were missing information on at least 1 of the predictors and were excluded from these models.
‡These SNPs were associated with FVIII activity in EA males as individual and in haplotypes.

**Discussion**

Using the ARIC database, we analyzed 19 FVIII SNPs and haplotypes for their association with FVIII activity and FVIII-VWF ratio. We also examined a cross-genetic influence of VWF SNPs that we have recently studied on FVIII activity. Consistent with a previous report, FVIII activity varied significantly among subjects (Figure 1) and was higher in AA subjects than EA subjects, but the FVIII-VWF ratio was comparable between the 2 ethnic groups (Table 1). This difference may be in part attributed to a higher BMI and increased prevalence of diabetes and hypertension in AA subjects (supplemental Figure 2A) because these covariates are known to associate with inflammation, a condition that increases secretion of acute-phase reactants such as FVIII and VWF. Women had significantly higher FVIII activity and a greater FVIII-VWF ratio compared with men. Age, race, and diabetes had the strongest influence on variations of FVIII activity, whereas the influence of BMI and sex was smaller, but significant (supplemental Figure 4). ABO (defined as O type vs non-O type) accounted for the strongest influence on variations of FVIII activity, whereas the influence of BMI and sex was smaller, but significant (supplemental Figure 4). ABO (defined as O type vs non-O type) accounted for

First, it has been reported that FVIII varies with VWF to keep its ratio relatively constant over a wide range of VWF levels. When analyzed in quartiles, we did find that 39%-66% of subjects had FVIII activities in parallel with VWF levels (Figure 2). However, this parallel distribution between the 2 measurements was not detected in up to 61% of subjects. Among them, ~2% subjects had either a very high VWF level paired with a very low FVIII or a very high FVIII paired with a very low VWF. This extreme split was consistently observed (1%-3%) when data were analyzed by race and sex (supplemental Figure 3), suggesting that FVIII and VWF ratio varies among individuals considerably more than previously reported. This extreme split may be explained by variable rates of synthesis of the 2 factors, different association kinetics between FVIII, and assay variations. Whether VWF multimer size and conformation affect its association with FVIII is not known. This split may be physiologically significant because the FVIII-VWF ratio is used to distinguish accelerated VWF clearance found in a subset of patients with VWD.

Second, we identified 4 FVIII SNPs that were significantly associated with FVIII activity after adjustment for age, sex, BMI, smoking status, diabetes, hypertension, and ABO genotypes (Table 3). These positive SNPs are intronic and clustered in intron 11, 13, and 14 that flank exons encoding A2 and B domains of the FVIII
Table 6. Difference (and P value) in adjusted mean FVIII activity (%) by copy numbers of the haplotype and by ABO adjustment for EA men (N = 3671)

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>ABO adjusted</th>
<th>P</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Block 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>No</td>
<td>$6 \times 10^{-4}$</td>
<td>3.74</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>$2 \times 10^{-4}$</td>
<td>3.89</td>
</tr>
<tr>
<td>TC</td>
<td>No</td>
<td>$7.7 \times 10^{-3}$</td>
<td>-3.20</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>$7.3 \times 10^{-3}$</td>
<td>-3.05</td>
</tr>
<tr>
<td>AC</td>
<td>No</td>
<td>$8 \times 10^{-2}$</td>
<td>-2.95</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>$2.9 \times 10^{-2}$</td>
<td>-3.50</td>
</tr>
<tr>
<td><strong>Block 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TACCCAC</td>
<td>No</td>
<td>$9.5 \times 10^{-3}$</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>$1.2 \times 10^{-3}$</td>
<td>2.71</td>
</tr>
<tr>
<td>GACCTT</td>
<td>No</td>
<td>$1.5 \times 10^{-3}$</td>
<td>-4.71</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>$2 \times 10^{-4}$</td>
<td>-5.24</td>
</tr>
<tr>
<td>GACACC</td>
<td>No</td>
<td>$9.2 \times 10^{-1}$</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>$6.5 \times 10^{-1}$</td>
<td>1.11</td>
</tr>
<tr>
<td>GACCTC</td>
<td>No</td>
<td>$9.8 \times 10^{-1}$</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>$8.2 \times 10^{-1}$</td>
<td>0.79</td>
</tr>
</tbody>
</table>

EA indicates Americans of European descent.

*Adjusted for age, BMI, diabetes, hypertension, and ever smoking.

Heavy chain. The effect sizes of these intronic SNPs are smaller, but often additive. Interestingly, these SNPs were found to 
associate with FVIII activity only in EA men, even though they 
were also detected in EA women and AA subjects. These 4 positive 
SNPs were also associated with the FVIII-VWF ratio in EA men 
and 4 additional SNPs were associated with the ratio in EA men 
and women. In addition, 2 major haplotype boxes were identified from 
these 19 SNPs and counted for 73.5%-83.6% of all 
women. In addition, 2 major haplotype boxes were identified from 
these 19 SNPs and counted for 73.5%-83.6% of all 
women. In addition, 2 major haplotype boxes were identified from 
these 19 SNPs and counted for 73.5%-83.6% of all 
women. In addition, 2 major haplotype boxes were identified from 
these 19 SNPs and counted for 73.5%-83.6% of all 
women. In addition, 2 major haplotype boxes were identified from 
these 19 SNPs and counted for 73.5%-83.6% of all 
women.

Table 7. Adjusted mean of FVIII activity (%) by VWF SNPs

<table>
<thead>
<tr>
<th>VWF SNP</th>
<th>ABO adjusted</th>
<th>FVIII activity for VWF genotype, mean (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td><strong>EA women, N = 4282†§</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS216315</td>
<td>No</td>
<td>126.50 (0.54)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>126.53 (0.51)</td>
</tr>
<tr>
<td>RS216318</td>
<td>No</td>
<td>126.51 (0.54)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>126.54 (0.51)</td>
</tr>
<tr>
<td>RS216299</td>
<td>No</td>
<td>126.30 (0.55)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>126.42 (0.51)</td>
</tr>
<tr>
<td>RS216295</td>
<td>No</td>
<td>126.34 (0.55)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>126.42 (0.51)</td>
</tr>
<tr>
<td>RS216298</td>
<td>No</td>
<td>126.32 (0.54)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>126.43 (0.51)</td>
</tr>
<tr>
<td><strong>EA men, N = 3774†</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS1063857</td>
<td>No</td>
<td>118.24 (0.83)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>118.50 (0.78)</td>
</tr>
<tr>
<td>RS723190†§</td>
<td>No</td>
<td>120.42 (0.56)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>120.36 (0.53)</td>
</tr>
</tbody>
</table>

SNP indicates single nucleotide polymorphism; and EA, Americans of European descent.

*Adjusted for age, BMI, diabetes, hypertension, and ever-smoking status.

†Due to small counts, AB genotype was combined with the minor BB genotype.

§All SNPs except RS723190 were previously associated with VWF Ag.31
In summary, we have found considerable variation in FVIII activity and FVIII-VWF ratios among the ARIC subjects. Four and 8 FVIII SNPs from a pool of 19 were associated with FVIII activity and FVIII-VWF ratio, respectively. We also identified 2 major haplotype blocks associated with FVIII activity. Strikingly, these associations were primarily found EA men, with some in EA female, but none in AA subjects. The impact of sex and race on FVIII activity remains to be further investigated. The results suggest that these intronic SNPs could directly regulate FVIII expression or act as markers for its regulation.

Acknowledgments

The authors thank the staff and participants of the ARIC Study for their important contributions.

This work is supported by an Atherosclerosis Risk in Communities (ARIC) contract and by National Heart, Lung, and Blood Institute (NHLBI) grant HL71895. The ARIC Study is carried out as a collaborative study supported by NHLBI contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022.

References

26. Takahashi Y, Kalafatis M, Girma JP, Sewerin K, Andersson LO, Meyer D. Localization of a factor VIII binding domain on a 34 kilodalton fragment of
Influence of single nucleotide polymorphisms in factor VIII and von Willebrand factor genes on plasma factor VIII activity: the ARIC Study

Marco Campos, Ashley Buchanan, Fuli Yu, Maja Barbalic, Yang Xiao, Lloyd E. Chambless, Kenneth K. Wu, Aaron R. Folsom, Eric Boerwinkle and Jing-Fei Dong